The Effect of Acclimation to Hypoxia on Fish Sensitivity to Ammonia

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Abstract

Aquaculture is a rapidly growing field that has potential to help solve world issues such as food shortages and agricultural sustainability. Winterkill is a naturally occurring event in which ice accumulation on shallow ponds and lakes decreases photosynthesis and results in oxygen levels falling below the required level for aquatic organisms (hypoxia). In intensive fish culture eliminating unwanted predatory fish is essential for the success of nursery ponds. Winterkills are sporadic, unpredictable, and often do not result in a complete kill of predators (Magnuson *et al*. 1985). The purpose of this experiment with juvenile yellow perch (*Perca flavescens*) is to test the effect of ammonia toxicity in combination with winterkill conditions (hypoxia), and in turn test its viability as an inexpensive and ecologically safe way to clear ponds of fish. Two experiments, each consisting of two trials were run with three levels of dissolved oxygen; normoxia, (7.93 ± 0.39 mg/L), moderate hypoxia, (3.19 ± 0.36 mg/L) and extreme hypoxia (1.97 ± 0.11 mg/L). The fish were given 3 days to acclimate to tank conditions and different oxygen saturation levels prior to being exposed to ammonia (0.37±0.02 mg/L). The fish were monitored for 1 day, with behavioral changes (breathing rates, loss of body equilibrium) and mortalities documented. Results showed that hypoxic conditions had a direct effect on the fish's sensitivity to ammonia toxicity, with average mortality rates of 6.25 ± 10.2%, 93.8 ± 10.8%, and 100%±0% for normoxia, moderate hypoxia, and extreme hypoxia respectively. These results show that with the additional stress brought about by ammonia exposure, the hypoxic conditions typically

found during winterkill conditions become lethal. It suggests that ammonia is a viable chemical to be used for winterkill enhancement

Introduction

Ammonia is a commonly occurring compound in aquatic ecosystems, originating from decomposition of organic material and metabolic wastes. In nature, ammonia exists in two forms: Ionized (NH_4^+) which is less toxic, and unionized (NH_3) which is highly toxic. If unionized ammonia is able to build up in the environment, it can become chronically toxic to fish in doses as low as 0.06 mg/L (Durborow, 1992). The mechanisms of Ammonia toxicity are not fully understood. In the past, it was thought that deaths from ammonia toxicity were due to suffocation of the fish, because ammonia causes several histopathological changes in the gills (hemorrhaging, lamellar fusion, excess mucous buildup, etc.) (Jease, et Al. 2003). However, evidence now suggests that ammonia toxicity affects the nervous system. Typically, fish are able to excrete excess ammonia directly through their gills (Martine and Bornancin, 1989). If the concentration present is higher than their ability to process, the common response is to convert the unionized ammonia to less toxic forms, specifically glutamine. If ammonia levels rise past the toxic threshold, excess glutamine builds up in the fish's brain, the energy cost of the synthesis and break down of the glutamine starves the nerve cells of ATP, causing paralysis and eventually death (Randall and Tsui, 2002).

Although suffocation is no longer the suspected mechanism for ammonia toxicity, a correlation has been observed between ammonia related deaths and low dissolved oxygen levels in ponds, especially in instances of "winter kills", a naturally occurring phenomena where ice and snow

cover over ponds cut off photosynthesis and oxygen production ceases. Oxygen is further depleted by the decomposition of dead plant material (Johnson, 1965). Winterkill events occur most commonly in late winter in northern lakes, especially during harsher winters. This naturally occurring process has the potential to be utilized and enhanced as a method of clearing aquaculture ponds of fish in preparation for raising new broods. Use of ammonia to enhance winterkill conditions in rearing ponds could be a sustainable and affordable alternative to the use of piscicides such as rotenone, which is potentially carcinogenic, and can leach into sediments and pose environmental risks (DeMong *et al.* 2001).

The joint effect of ammonia and hypoxia on fish physiology is likely caused by ammonia's effect on hemoglobin's ability to combine with oxygen. This idea was first proposed by D.R. Brockway (1950) when the oxygen level in the blood of Rainbow Trout (*Oncorhynchus mykiss*) dropped to $1/7^{th}$ the normal value following ammonia exposure. This idea was further corroborated by Sousa and Mead (1977) when they discovered that ammonia causes an acidosis that interferes with hemoglobin's ability to combine with oxygen. It has been shown by several studies that hypoxia and ammonia toxicity are not independent. Magaud *et al.* (1996) determined that the survival probability of Rainbow Trout was drastically effected by the combination of the two effects when compared to hypoxia and ammonia stress tested independently. At concentrations of 1.7 mg/L dissolved O_2 and 0.5 mg/L dissolved NH_3 for a 30 minute period, survival probability was found to be 0.93 and 0.62 respectively. However, when the two stressors were combined, survival probability dropped to 0. A study conducted by Thurston *et al* (1981) also showed a strong correlation between decreased dissolved oxygen and increased susceptibility to ammonia toxicity, finding a correlation coefficient of 0.9346 between oxygen

level and LC50, where the LC50 was about ½ the level at 4.0 mg/L dissolved oxygen than at 8.0 mg/L

Materials and Methods:

The experiment conducted in the aquaculture laboratory at The Ohio State University. Six juvenile captive-raised Yellow Perch (*Perca flavescens*) were placed into 12 separate 40 Liter glass aquaria (4 replicates per treatment) in a semi-recirculating system. Water was cycled at a rate of 3.3 L/min to prevent buildup of metabolic wastes and to keep temperatures constant. The water source was Columbus City water that was passed through charcoal filters, then treated with sodium thiosulfate (4 mg/L) in order to keep chlorine levels below 0.1 mg/L.

Two separate tests with different ammonia concentrations were carried out in two trials each with the twelve aquaria separated into three groups of four, each pertaining to a different dissolved oxygen concentration: 1.97 ± 0.11 mg/L (severe hypoxia), 3.19 ± 0.36 mg/L (moderate hypoxia) and 7.93 ± 0.39 mg/L. Dissolved oxygen levels were adjusted to the desired treatment levels using an in-tank aeration column. Water entering each tank flowed through a 12”X2” PVC column at a rate of 0.3L/min. Gas was mixed with the water through countercurrent exchange. Water entered at the top of the column and gas (either nitrogen or air depending on the treatment) was injected at the bottom using an air stone. To improve gas exchange, the columns were packed with 7 X 10 mm plastic bio-substrate to increase the contact surface area of the incoming water. Atmospheric air was used in the columns to increase dissolved oxygen to desired levels for the normoxic treatments, and nitrogen was used to decrease dissolved oxygen to the desired levels for the severely hypoxic treatments. The incoming water used for

the moderately hypoxic treatments was not treated with gas, as it already had dissolved oxygen levels within the desired range (≈3.5 mg/L O_2). Oxygen levels were the same for both trials. Each trial was conducted using a different concentration of un-ionized ammonia. The first set of trials was conducted with an ammonia concentration of 0.25 mg/L, and the second set was conducted with a concentration of 0.35 mg/L. Ammonia was introduced in the form of ACS grade ammonium chloride (NH_4Cl), which was introduced into the tanks using a peristaltic pump at a rate to maintain ammonia levels based on the flow of incoming water.

The perch used for this experiment were from brood stock raised at The Ohio State University for two generations. Ambient water conditions, including temperature, pH, dissolved oxygen, ionized ammonia and unionized ammonia levels were monitored periodically throughout the duration of the experiment using a handheld multi-parameter instrument (YSI inc. Yellow Springs, Ohio). The mean levels of these are listed in table 1. The fish were given three days to acclimate to the conditions of the tanks before the addition of ammonia began. Once the ammonia exposure began, the fish were monitored every hour for hours 1-10, and then again from hours 22-24 with changes in behavior noted, and dead fish removed in the case of mortality. Mortality differences were compared using one way ANOVA. When statistical differences were found ($p<0.05$), post-hoc analysis was carried out using the Tukey HSD method. Two-way ANOVA was used to analyze mortality differences between different ammonia concentrations at different oxygen concentrations. Ambient water conditions were also tested hourly during this period to ensure they remained constant. The fish were not fed during the duration of the experiment. After 24 hours, ambient water conditions were allowed to return to normal.

Table 1: Mean ambient water conditions of the tanks throughout the duration of the experiment. All figures measured in mg/L.

	Temperature	DO	pH	NH_4^+	NH_3
Hypoxia	14.07 ± 0.12	1.97 ± 0.13	7.20 ± 0.01	90.06 ± 2.73	0.37 ± 0.04
Moderate	14.35 ± 0.24	3.19 ± 0.42	7.16 ± 0.01	89.22 ± 1.86	0.36 ± 0.01
Normoxia	14.23 ± 0.16	7.93 ± 0.45	7.20 ± 0.03	88.76 ± 2.25	0.38 ± 0.03
Hypoxia	11.24 ± 0.15	1.72 ± 0.11	7.23 ± 0.04	64.71 ± 0.24	0.23 ± 0.02
Moderate	11.54 ± 0.40	2.62 ± 0.13	7.16 ± 0.01	64.16 ± 0.46	0.20 ± 0.01
Normoxia	11.30 ± 0.10	8.90 ± 0.26	7.28 ± 0.01	64.99± 1.90	0.26 ± 0.01

Respiration rate was monitored by counting the number of opercular movements of the fish per minute at three different intervals: before ammonia exposure, during exposure, and 24 hours after exposure. The mean values of these observation were compared.

Results

Survivorship varied widely across experimental conditions. For treatment 1 (NH_3=0.35 mg/L), mortalities began to occur under severely hypoxic within two hours, and eventually reached 100% ± 0. Under moderately hypoxic conditions, mortalities began to occur within four hours, and eventually reached 93% ± 13 by the end of the 24 hour exposure period. The first mortalities did not occur until the overnight period of the exposure and were discovered the following morning. Mortalities at this oxygen level eventually reached 7% ± 13 by the end of the 24 hour exposure period.

For treatment 2 (NH_3=0.25 mg/L) mortalities began much later. For the normoxic treatment, the first mortalities under severely hypoxic conditions began to occur after four hours, and eventually reached 75% ± 49. Under moderately hypoxic conditions mortalities began after nine hours, and eventually reached 31% ± 23 within the 24 hour exposure period. Under normoxic conditions for this treatment no mortalities occurred due to ammonia exposure. The only fish to die during this treatment became trapped between the PVC column and the wall of the tank, was unable to draw water across its gills and subsequently suffocated.

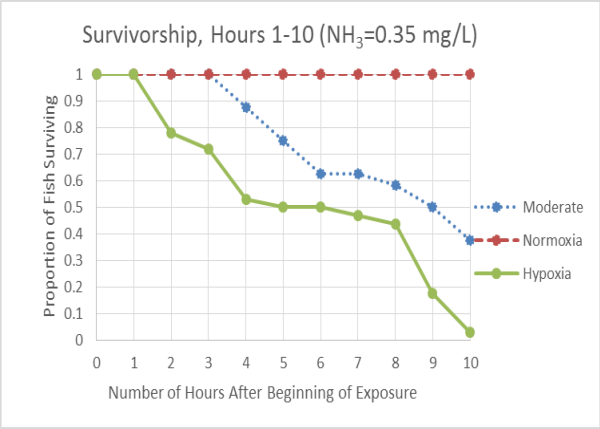

Figure 1: Survivorship of fish under different oxygen concentrations when dissolved ammonia concentration= 0.35 mg/L

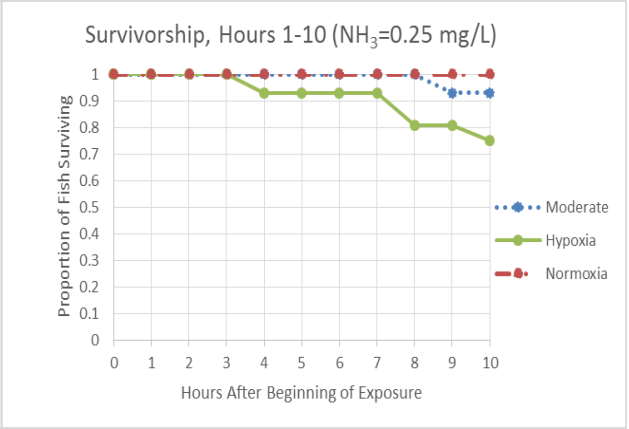

Figure 2: Survivorship of fish under different oxygen concentrations when dissolved ammonia concentration= 0.35 mg/L

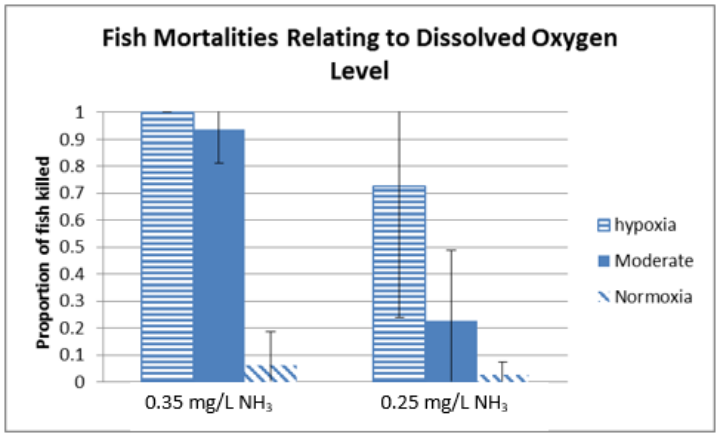

Figure 3: Total mortalities resulting from ammonia exposures of different concentrations for different dissolved oxygen levels over 24 hours. Statistical significance was found between mortalities under severely hypoxic conditions and normoxic conditions at both ammonia concentrations. Significance was also observed between the moderately hypoxic group and the severely hypoxic group when NH_3=0.35 mg/L. Significance was also observed between mortalities of the moderately hypoxic groups at different NH_3 levels.

ANOVA tests found a significant variance for test one when NH_3 = 0.35 mg/L (p= 0.034). Post hoc analysis was carried out using the Tukey HSD method, and found significant variance in mortalities between the severely hypoxic groups and both the moderately hypoxic and normoxic treatments (p <0.001 for both). Significant variance was also found (p=0.029) for the second test when NH_3 = 0.25 mg/L. Post hoc analysis of these results showed a significant difference between mortalities under severely hypoxic conditions and mortalities under normoxic conditions (p=0.021). Two way ANOVA tests conducted between the two ammonia concentrations also showed significant results (p=0.022). Post hoc analysis of this observation showed a significant difference between the mortalities of the two moderately hypoxic treatments at different ammonia concentrations (p=0.031).

Average number of opercular movements at the different stages of the experiment are shown in figure 4. Average number of movements was shown to be much lower during the post exposure period for fish that had been exposed to lowered oxygen levels, whereas there was no significant difference observed at any stage for the fish in normoxic conditions.

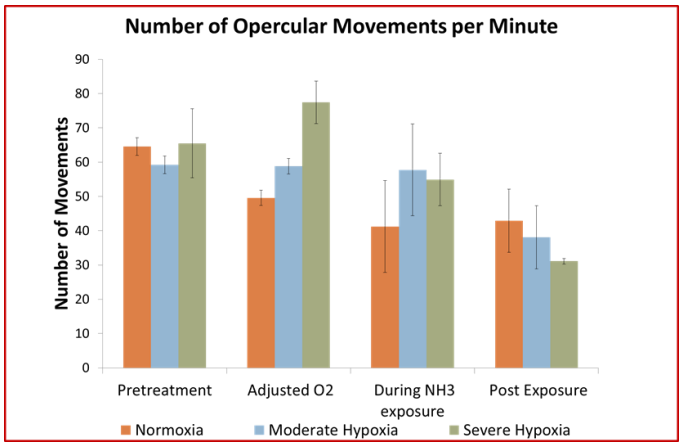

Figure 4: Average number of opercular movements per minute measured at four different stages of the experiment

Discussion:

Congruent with the findings of several studies (Lloyd, 1961; Magaud, 1997; Merkins, 1957; Randall and Tsui, 2002; Thurston *et al*. 1981) a significant relationship was found between fish susceptibility to ammonia toxicity and dissolved oxygen level. This relationship is strongly illustrated by the differences between mortality levels of moderate and severe hypoxia at the different ammonia levels, in which lethality rose by more than a factor of ten. While there was

no statistically significant difference between the two groups at 0.35 mg/L ammonia, the difference in oxygen was enough for a significant decrease in mortality percentage for fish in the moderately hypoxic conditions at 0.25 mg/L ammonia. This relationship shows promising potential to be utilized in aquaculture to enhance winterkills to clear ponds of undesired fish. Studies on the implementation of this tactic in the United States Midwest began in 2011. (Gaikowski, 2011).

Throughout various times in the experiment the fish's respiration rate was measured by counting the number of movements of their opercula per minute. The times tested were before the experiment began (pretreatment), after adjusting the dissolved oxygen levels, during the ammonia exposure, and after the exposure during recovery. Predictably, the fish in lower levels of oxygen had more opercular movements than the fish exposed to higher levels of oxygen. During the exposure, respiration decreased. This is most likely due to irritation of the gills caused by the ammonia (Jease *et al*. 2003). Opercular movements decreased even further during the recovery period after exposure. This could likely be due to the fish becoming acclimated to their lower oxygen levels. This idea is supported by the fact that the sharpest drop in opercular movements is seen in the fish exposed to severe hypoxia, further supporting findings that suggest cause of death is not due to suffocation (Randall and Tsui, 2002; Sousa and Meade, 1977). Supporting the idea that death is caused by neurological damage, fish displayed symptoms such as erratic movements and paralysis during exposure. Some surviving fish never fully recovered, and continued to display partial paralysis.

Conclusion

The relationship between dissolved oxygen level and fish susceptibility to ammonia toxicity observed during this study and past studies shows promising potential for application in the aquaculture industry. The fact that ammonia does not accumulate in sediments and does not persist for extended periods in water makes it a more sustainable choice than traditional piscicides. Also, the fact that ammonia is non-toxic to humans give further support to its potential for aquacultural use.

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Key Words: Ammonia toxicity, hypoxia, yellow perch, *Perca flavescens*, Winterkill Enhancement, Dissolved Oxygen.

References:

Austin, Milton, *et. al*. 1996. Ohio Pond Management Handbook Division of Wildlife, Ohio Department of Natural Resources

Avella, Martine, and Michel Bornancin. "A new analysis of ammonia and sodium transport through the gills of the freshwater rainbow trout (Salmo gairdneri)." Journal of Experimental Biology 142.1: 155-175.

Brockway, Donald R. 1950. "Metabolic products and their effects." The Progressive Fish-Culturist 12.3: 127-129.

DeMong, Leo, *et al*. 2001. Rotenone in Fisheries: Are the Rewards Worth the Risk. Bethesda, Maryland: American Fisheries Society.

Durborow, R.M., Crosby, D.M., and Brunson, M.W. 1992. Ammonia in Fish Ponds. Journal of the Fisheries Research Board of Canada. 32:2379-2383

Gaikowski, Mark P. 2011. "Assessment of Carbon Dioxide (CO2) and Inorganic Nitrogen Compounds to Enhancement Winter Kill in Natural Rearing Ponds Used For Fish Production in the North Central Region." Ncrac.org. U.S. Geological Survey Upper Midwest Environmental Sciences Center. Web. 15 Oct. 2014.

Jease, H.M. *et. al.* 2003. Structural Changes in Gills of Lost River Suckers Exposed to elevated pH and Ammonia Concentrations. Comparative Biochemistry and Physiology Part C, 134. 491-500

Johnson, M.G. 1965 .Limnology of Ontario Ponds in Relation to Winterkill of Largemouth Bass.

The Progressive Fish-Culturist, 193-198

Lloyd, Richard. "Effect of dissolved oxygen concentrations on the toxicity of several poisons to rainbow trout (Salmo gairdnerii Richardson)." Journal of Experimental Biology 38.2 (1961): 447-455.

Magaud, Hélène, et al. "Modelling fish mortality due to urban storm run-off: interacting effects of hypoxia and un-ionized ammonia." Water Research 31.2 (1997): 211-218.

Magnuson, John J., et al. 1985. Surviving winter hypoxia: behavioral adaptations of fishes in a northern Wisconsin winterkill lake. Environmental Biology of Fishes 14.4: 241-250.

Merkins, J.C. and Downing, K.M. 1957. The effect of Tension of Dissolved Oxygen on the Toxicity of un-ionized Ammonia to Several Species of Fish. Annals of Applied Biology. 45(3) 521- 527.

Randall, D.J. and Tsui, T.K.N. 2002. Ammonia Toxicity in Fish. Marine Pollution Bulletin 45(1-12):17-33

Sousa, Robert J., and Thomas L. Meade. 1977. "The influence of ammonia on the oxygen delivery system of coho salmon hemoglobin." Comparative Biochemistry and Physiology Part A: Physiology 58.1: 23-28.

Thurston, R.V., Phillips, G.R., Russo, R.C., and Hinkins, S.M. 1981. Increased Toxicity of Ammonia to Rainbow Trout (salmo gairdneri) Resulting from Reduced Concentration of Dissolved Oxygen. Canadian Journal of Fisheries and Aquatic Sciences. 38: 983-988